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(57) Abstract

An inhibitor of protein tyrosine phosphatase α is useful in the treatment of a tumour exhibiting an elevated level of $pp60^{c\text{-}src}$ kinase activity. The inhibitor may therefore be used in the treatment of human colon carcinoma.

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INHIBITORS OF PROTEIN TYROSINE-PHOSPHATASE α FOR TREATMENT OF TUMORS.

This invention relates to the treatment of tumours, in particular tumours exhibiting an el vated level of $pp60^{c\text{-}src}$ kinase activity such as human colon carcinoma.

The kinase activity of $pp60^{c\text{-}src}$ is specifically and transiently increased during mitosis and repressed during interphase[1]. Loss of cell cycle control of $pp60^{c\text{-}src}$ occurs upon mutation of Tyr 527 to Phe or when $pp60^{c\text{-}src}$ is associated with polyoma middle-T-antigen, and these conditions result in cell transformation or tumourigenesis [2,3]. In both cases $pp60^{c\text{-}src}$ has elevated kinase activity which is maintained throughout the cell cycle and accompanied by dephosphorylation of the carboxy-terminal negative regulatory [4-7] Tyr 527 site, or mimicry of Tyr 527 dephosphorylation in the case of the mutant.

Protein tyrosine phosphatases (PTPases) have been considered as potential anti-oncogenes [8, 9], where mutations giving rise to a non-functional gene product or gene deletion could result in tumourigenesis. We have now found that over-expression of the receptor-like human PTPα (HPTPα) results in persistent activation of $pp60^{c\text{-}src}$ with concomitant cell transformation and tumourigenesis. This indicates instead that PTPα may function as an oncogene and not an anti-oncogene.

According to the present invention, there is provided an inhibitor of PTPα for use in the treatment of a tumour exhibiting an elevated level of $pp60^{c\text{-}src}$ kinase activity.

PTPases may be more fully termed as protein tyrosine phosphate phosphohydrolases (EC 3.1.3.48). They may be divided into three classes based on their structural organisation. Class I contains the low molecular mass non-receptor molecules

possessing a single catalytic domain (PTP IB, TCPTP, rat brain PTP). Class II and II PTPases are receptor-like transmembrane proteins. The sole member of Class II is human PTPβ (HPTPβ) which has a single cytoplasmic catalytic domain. Class III members are LCA, LAR, HPTPα, HPTPγ, HPTPδ, HPTPε, DPTP, DLAR and possess two repeated putative catalytic domains in the cytoplasmic region of the molecule.

Inhibitors of PTPα include chemical compounds, for example zinc ions, vanadates such as sodium orthovanadate and arsenites such as phenylarsine oxide. These compounds are, however, fairly toxic. Non-toxic inhibitors are preferred. The inhibitor may therefore be an antibody, polyclonal or monoclonal. The antibody is typically an inhibitory antibody to the extracellular domain of PTPα or to the active site in the catalytic domain of PTPα.

Inhibitors such as zinc, vanadate, arsenite and antibody act directly on PTPα. However, inhibition may be achieved in other ways. Inhibition of endogenous PTPα may be effected by gene transfer of a mutant PTPα cDNA where the mutant protein product interacts with and blocks endogenous PTPα activity or competes with endogenous PTPα for activating proteins, ligands, etc. A suitable cDNA encodes a mutant PTPα in which the Cys residues of the catalytic domain(s) have been mutated to Ser.

The inhibitors are used to treat a tumour exhibiting an elevated level of $pp60^{c\text{-}src}$ kinase activity. Any tumour which has abnormally active or overactive $pp60^{c\text{-}src}$, which may be a result of PTPα overexpression or overactivation in the tumour, may be treated. The tumour may be a tumour with increased $pp60^{c\text{-}src}$ activity which cannot be accounted for by a

proportional increase in $pp60^{c\text{-}src}$ amount. Such tumours include human colon carcinoma, rhabdomyosarcoma, osteogenic sarcoma and Ewing's sarcoma [10-14]. The inhibitors may be particularly used in the treatment of human colon carcinoma which is the third most common human malignancy. The inhibitors may therefore be used in the treatment of colorectal cancer.

An agent for use in the treatment of a tumour exhibiting an elevated level of $pp60^{c\text{-}src}$ kinase activity can therefore be provided. The agent comprises a HPTPα inhibitor. A pharmaceutical composition may be formulated, comprising the inhibitor and a pharmaceutically acceptable carrier or diluent.

A chemical or antibody inhibitor may be given by injection. When an injectable preparation is produced, a pH controlling agent, a buffer, a stabilizing agent and/or an excipient may be added. Further, according to conventional techniques, lyophilized injectable preparations may be produced. There can be produced preparations for subcutaneous, intramuscular or intravenous injection.

A chemical inhibitor may alternatively be administered by the oral or rectal route. Orally administrable solid preparations may be produced by adding an excipient and, if desired, a binding agent, disintegrator, lubricating agent, coloring agent, and/or flavouring agent, and the like to the inhibitor, and forming tablets, coated tablets, granules, powders or capsules according to conventional techniques. Oral liquid compositions may be prepared by adding a flavouring agent, buffer, and/or stabilizer to the main drug and forming a syrup or dry syrup. A rectal suppository may be prepared by adding an excipient and, if desired, a surfactant to the

inhibitor to form the rectal suppository according to conventional techniques.

A DNA sequence encoding a mutant PTPα may be incorporated in a retroviral or adenoviral vector or in liposomes for the purpose of gene transfer[15]. The vectors may transfer the DNA into cultured human tumour cells which are administered to a patient by injection, for example intratumoural injection. Cells from the tumour it is wished to treat may therefore be removed from a patient, grown in culture, infected with the vector, selected for positive infection and then reintroduced into the patient. Liposomes containing DNA, for example plasmid DNA, are injected directly into tumour cells to accomplish gene transfer. In an alternative embodiment, physical DNA transfer may be achieved using biolistic technology i.e. a gene gun.

In use, a therapeutically effective amount of the inhibitor is administered to a host having a tumour exhibiting an elevated level of $pp60^{c\text{-}src}$ kinase activity. The host is typically a human but may be another mammal. The condition of a patient may thus be improved. The tumour from which a patient suffers may be ameliorated. This treatment may be given in conjunction with another method of treatment, for example surgery, to eradicate the tumour.

The dosage levels for the inhibitor differ depending on a variety of factors including condition of the patient, the age and body weight of the patient, the disease state being treated and the administration form. Dosage levels of the order of about 0.05-500 mg per day are useful for injection of a chemical or antibody inhibitor though the dosage levels may

differ from portion to portion. When a chemical inhibitor is administered orally or rectally, the dosage levels are about 0.05-1000 mg per day. The dosage levels may be changed according to the requirements of a doctor.

For the purpose of gene transfer, enough DNA is used to introduce at least one gene per target cell such as 1 to 10000 DNA molecules per cell. The amount of DNA used depends on the tumour mass and estimated number of cells per tumour. It also depends on the amount of endogenous PTPα per tumour cell. Generally an amount of inhibitory mutant PTPα DNA is introduced that gives expression of an excess amount of mutant PTPα protein.

The following Examples illustrate the invention. In the accompanying drawings:

Figure 1 shows overexpression of active PTPα. a, Western blotting with anti-PTPα antiserum of solubilised membrane (M) and cytosolic (C) fractions from parental cells (REF) and cells transfected with PTPα cDNA (α-7, α-21, α-29). b, PTPase specific activities of cytosolic (solid bars) and solubilised membrane (hatched bars) fractions measured towards the phosphotyrosyl-peptide RR-src. Fractions from control transfected cells (REF-neo) are designated as neo. c, PTPase activity of immunoprecipitated PTPα from parental REF (□), control transfected REF-neo (♦) and PTPα-transfected (●, α-7; ○, α-21; Δ , α-29) cells.

Figure 2 shows the morphologies of control REF-neo and PTPα-overexpressing cells. a, Subconfluent and b, four day post-confluent cells in DME medium with 10% fetal calf serum (FCS) (320X). c, 24 h after changing to Dulbecco's minimum

essential (DME) medium with 0.1% FCS (320X). d, 72 h after changing to DME medium with 0.1% FCS (320X). e, C lonies on soft agar with serum-free DME medium (32X).

Figure 3 shows the protein kinase activity, immunoblotting and cyanogen bromide phosphopeptide analysis of $pp60^{c\text{-}src}$. Cells maintained in medium with 0.1% FCS for 48 h were harvested (0 h) or treated with 10% FCS for 8 h prior to harvesting (8 h). Lysates of control REF-neo cells (neo) and PTPα-overexpressing (α-7, α-21, α-29) cells were equalised for protein and immunoprecipitated with anti-src monoclonal antibody 327. a, Top; A portion of each immunoprecipitate was incubated with the exogenous substrate enolase and [γ-^{32}P]ATP and the mixture resolved on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography. Bottom; Another portion of each immunoprecipitate was run on 10% SDS-PAGE, immunoblotted and probed with antibody 327 to measure $pp60^{c\text{-}src}$ levels. b, Cyanogen bromide phosphopeptides of immunoprecipitated $pp60^{c\text{-}src}$ from lysates of cells maintained in medium with 0.1% FCS for 48 h and then metabolically labelled with ^{32}P-orthophosphate for 16 h. c, as in b except that $pp60^{c\text{-}src}$ immunoprecipitated from quiescent control REF-neo cells was incubated without (minus) or with (plus) the bacterially expressed and purified intracellular region of PTPα prior to digestion with cyanogen bromide. In b and c, the position of the 32 kDa phosphopeptide is designated by the open arrow and the position of the 4 kDa phosphopeptide containing Tyr 527 is designated by the closed arrow.

Example 1

1. To facilitate cloning of the full-length PTPα cDNA[16] into the BamH1-HindIII site of the expression vector pXJ41[17], a 570 bp fragment of the 5′ portion of the gene was first amplified by PCR. A BamH1 site (GGATCC) and an optimal Kozak consensus sequence (CCACC) were added to the 5′ oligonucleotide (5′-AAGGATCCAACCACCATGGATTCCTGG-3′) and the 3′ oligonucleotide contained in the unique Ec1XI restriction site in the PTPα cDNA. The fidelity of amplification was confirmed by sequencing.

The PCR fragment was cleaved with BamH1 and Ec1XI and ligated with a Ec1XI-HindIII fragment containing the rest of the PTPα coding sequence, and this was inserted into a BamH1-HindIII site in the expression vector pXJ41 to give pXJ41-PTPα. The neo gene in a pMAM-neo vector (Pharmacia) was excised with BamH1 and blunt-ended by filling with Klenow. It was inserted into the pXJ41-PTPα and pXJ41 vectors which had been cut at a Sa1I site (downstream of the PTPα insert in the former) and likewise blunt-ended. The REF cells (about 10^6 per 15 cm dish) were transfected with 20 μg of either pXJ41-PTPα-neo or pXJ41-neo expression vector using the calcium phosphate method [18].

After 16 h the cells were washed with DME medium (containing 4.5 g/l glucose and penicillin/streptomycin) and incubated with DME medium containing G418 (500 μg/ml) and 10% FCS. The medium was changed every four days, and colonies were isolated after 21 days. To prepare cell membrane and cytosolic fractions, confluent cells were rinsed twice with cold phosphate-buffered saline, scraped into harvesting buffer (50mM Tris-Cl pH 7.6, 150mM NaCl, 2mM PMSF, 10 μg/ml aprotinin) and

pelleted by low speed centrifugation. After resuspension of the pellets in harvesting buffer and brief sonication to lyse th cells, the lysates were centrifuged at 100,000xg for 30 min at 4°C to obtain the sytosolic supernatants.

The pellets were rinsed once with harvesting buffer, resuspended in harvesting buffer with 1% Triton X-100 and solubilised on ice for 60 min. After centrifugation at 100,000xg as above the supernatants comprised the solubilised membrane fractions. For Western blotting, 150 μg protein from each fraction was electrophoresed on 7.5% SDS-PAGE and transferred to nitrocellulose membranes in 50mM Tris base/0.56% glycine/10% methanol (80V, 75 min, 25°C). The membranes were probed with a 1:500 dilution of anti-PTPα antiserum (raised in rabbits against a peptide comprising the carboxy-terminal amino acid sequence IDAFSDYANFK of PTPα) followed by a 1:3000 dilution of goat anti-rabbit IgG conjugated to peroxidase (Sigma), and developed using the ECL system (Amersham).

PTPase specific activity was measured at 30°C in reactions containing 50mM Mes pH 6.0, 0.5 mg/ml bovine serum albumin (BSA), 0.5mM dithiothreitol (DTT), (5μM phosphotyrosyl-RR-src peptide (RRLIEDAEY(P)AARG, corresponding to the sequence encompassing Tyr 416 of $pp60^{c\text{-}src}$ and phosphorylated as described[16]) and 3.3 μg/ml solubilised membrane protein or 6.6 μg/ml cytosolic protein. Reactions were linear over the times assayed. For immunoprecipitation of PTPα, confluent cells were lysed in harvesting buffer with 1% Triton X-100 and centrifuged at 100,000xg for 30 min.

Anti-PTPα antiserum (150 μl) was added to 6 ml of 2 mg/ml supernatant and mixed at 4°C for 16 h. Protein A-Sepharose

(Pharmacia) was added and mixed at 4°C for 2 h. The immunoprecipitated pellets obtained by centrifugation were washed three times with 50mM Tris-Cl pH 7.6, 0.5M NaCl, 1mM PMSF, 10 μg/ml aprotinin and packed into small columns in the same buffer. Protein was eluted from the beads with 0.6 ml 0.1M glycine, pH 2.5 and immediately neutralised with 20 μl 1M Tris-Cl, pH 8.0. The results are shown in Figures 1 and 2 and Table 1.

2. Cells were lysed in RIPA buffer[19] (2 ml/15 cm dish) and the lysates centrifuged at 100,000xg for 30 min. Anti-src monoclonal antibody (Mab 327, Oncogene Science) was added to the lysates (1 μg/2ml) and mixed at 4°C for 1 h. Goat anti-mouse IgG (Sigma) was added (4 μg/2 ml) and mixed as above, followed by a one-tenth volume of Protein A cell suspension (Sigma) and mixing as above. After low speed centrifugation the immunoprecipitates were washed as described[19].

Immunoblot analysis of immunoprecipitated $pp60^{c\text{-}src}$ levels was as described in Example 1 except immunoblots were probed with a 1:1000 dilution of 0.1 mg/ml Mab 327 followed by goat anti-mouse IgG conjugated to peroxidase (1:1000) (Sigma). The kinase activity of immunoprecipitated $pp60^{c\text{-}src}$ was determined in 20 μl reactions containing 10mM Pipes, pH 7.0, 5mM $MnCl_2$, 0.5mM DTT, 2.5 μg acid-treated enolase and 10 μCi [γ-^{32}P]ATP at 37°C for 5 min. For cyanogen bromide phosphopeptide analysis, cells were labelled for 16 h with 1.5 mCi/ml ^{32}P-orthophosphate (Amersham) in phosphate-free DME containing normal DME (95:5, vol:vol) and 0.1% FCS.

Immunoprecipitates of $pp60^{c\text{-}src}$, prepared from the various cells as just described, were electrophoresed on 10% SDS-PAGE

and the position of ^{32}P-labelled pp60$^{c\text{-}src}$ determined by autoradiography. Corresponding pieces of the gel were cut out and electroeluted in 25mM Tris, 192mM glycine, 0.1% SDS, 2% 2-mercaptoethanol at 90V for 2 h. Electroeluted protein was precipitated with 10% trichloroacetic acid with 100 μg bovine serum albumin as carrier. The precipitate was washed once with cold ethanol, dried, digested with cyanogen bromide and analysed on 20% SDS-PAGE as described[20]. For in vitro treatment of pp60$^{c\text{-}src}$ with PTPα prior to cyanogen bromide digestion, ^{32}P-labelled pp60$^{c\text{-}src}$ immunoprecipitates were washed four times with 50mM Mes, pH 6.0, 0.5mM DTT and incubated at 30°C for 60 min in the same buffer with 0.5 μg/ml bovine serum albumin in the presence or absence of 15 μg of PTPα (intracellular domain)[16]. The results are shown in Figure 3.

3. Fischer rat embryo fibroblasts (REF) were transfected with the full length human receptor-like protein tyrosine phosphatase α (PTPα) cDNA in an expression vector driven by the human cytomegalovirus promoter and G418-resistant cell lines were isolated. Western blotting with anti-PTPα antibodies detected elevated levels of a protein of an expected[21] M_r of about 130 kDa in total lysates of several cell lines (not shown), and three lines (α-7, α-21, α-29) were selected for further analysis.

In accord with the transmembrane nature of PTPα solubilized membrane fractions from these cells contained much higher amounts of PTPα than those from parental cells (REF), and the overexpressed PTPα was predominantly localized to the membrane although some was detectable in the cytosolic fraction (Fig. 1a). The membrane PTPase specific activities were

elevated 3-fold over those in parental and control cells transfected with expression plasmid lacking PTPα cDNA (REF-neo), while cytosolic PTPase specific activities were increased only 1.5-fold (Fig. 1b). Immunoprecipitates of PTPα from α-7, α-21 and α-29 cell lysates also had higher phosphatase activity than those prepared from control or parental cell lysates (Fig. 1c), demonstrating that the observed increases in PTPase specific activities are due to PTPα overexpression.

At subconfluency, the PTPα-overexpressing cells had a fibroblastic phenotype (Fig. 2a). At this stage the α-21 cells were beginning to exhibit a transformed phenotype with some of the cells appearing rounded and highly refractile. In contrast to the control cells, none of the PTPα-overexpressing cell lines were growth arrested through contact inhibition (Table 1). They grew in multilayers and formed foci containing refractile and disordered cells (Fig. 2b). The α-7, α-21 and α-29 cells also had a reduced requirement for serum growth factors as evidenced by their continued proliferation in medium with 0.1% FCS, whereas control cells became quiescent (Table 1).

In particular, a high proportion of α-21 cells appeared transformed within 24 h of serum deprivation (Fig. 2c). The α-7 and α-29 cells likewise appeared transformed after the concentration of FCS in the medium was reduced from 10% to 0.1%, although this phenotypic change was more apparent after 48-72 h (Fig. 2d). All three PTPα-overexpressing cells exhibited anchorage-independent growth in soft agar (with α-7 and α-21 cells showing enhanced colony formation in the absence of serum) (Fig. 2e and Table 1) and the ability to form tumours

in nude mice with a short latency period (Table 1). The above features of these cells in culture in conjunction with th ir demonstrated in vitro tumorigenicity indicates that PTPα has transforming capability and may be oncogenic when overexpressed.

A few enzymes are activated by tyrosine dephosphorylation, such as the src family tyrosine kinases[4, 22-24] and the cell cycle serine/threonine kinase $p34^{cdc2}$ [25-27]. Of these, certain mutant forms of $pp60^{c\text{-}src}$, including its oncogenic counterpart $pp60^{v\text{-}src}$, have transforming ability[5-7, 28]. The activity of $pp60^{c\text{-}src}$ in the control REF-neo and PTPα overexpressing cells was tested by immunoprecipitating $pp60^{c\text{-}src}$ and measuring its ability to phosphorylate the exogenous substrate enolase. In cells maintained in medium with 0.1% FCS for 48 h, the kinase activity of $pp60^{c\text{-}src}$ from α-7, α-21 and α-29 cells was increased 3- to 6-fold over that of $pp60^{c\text{-}src}$ from control cells, and the autophosphorylation activity of $pp60^{c\text{-}src}$ was also higher (Fig. 3a, top).

A similar activation of $pp60^{c\text{-}src}$ was observed in cells maintained as above but treated with 10% FCS for 8 h (Fig. 3a, top) or 24 h (not shown). The amounts of $pp60^{c\text{-}src}$ protein immunoprecipitated from all cells were comparable, as determined by probing immunoblots with an anti-src monoclonal antibody (Fig. 3a, bottom). Thus the higher activity of $pp60^{c\text{-}src}$ in the PTPα-overexpressing cells is not due to a corresponding increase in the level of $pp60^{c\text{-}src}$ protein.

In many instances, activation of $pp60^{c\text{-}src}$ involves dephosphorylation of Tyr 527[4-7, 29]. To determine whether the activation of $pp60^{c\text{-}src}$ in PTPα-overexpressing cells could be

accounted for by dephosphorylation of this residue, we immunoprecipitated pp60$^{c\text{-}src}$ from ^{32}P-labelled cells and analysed its cyanogen bromide phosphopeptides. A 32 kDa phosphopeptide containing amino-terminal sites for serine and threonine phosphorylation[20, 30] was recovered from cleaved pp60$^{c\text{-}src}$ from both control (REF-neo) and PTPα-overexpressing cells (Fig. 3b, open arrow).

However, the pp60$^{c\text{-}src}$ from control REF-neo cells was phosphorylated at Tyr 527 (represented by a 4 kDa phosphopeptide[20]), while no phosphorylation of Tyr 527 was detected in pp60$^{c\text{-}src}$ from α-7, α-21 and α-29 cells (Fig. 3b, closed arrow). A direct interaction between PTPα and pp60$^{c\text{-}src}$ could be demonstrated in vitro, where bacterially expressed and purified PTPα was able to dephosphorylate Tyr 527 of pp60$^{c\text{-}src}$ immunoprecipitated from quiescent control cells (Fig. 3c).

The activation state of pp60$^{c\text{-}src}$ reflects the balance of opposing activities of a Tyr 527 kinase and phosphatase, where phosphorylation of Tyr 527 negatively regulates pp60$^{c\text{-}src}$ activity. A specific Tyr 527 kinase has recently been identified and cloned[31, 32], although as yet nothing is known of the regulation of its activity. Cell cycle-regulated inhibition of such a kinase or activation of an appropriate tyrosine phosphatase, or both, could result in Tyr 527 dephosphorylation and activation of mitotic pp60$^{c\text{-}src}$. The unregulated occurrence of these events may lead to uncontrolled cell proliferation. In accord with this, we have shown that the constitutive overexpression of active PTPα subverts the normal control of cell growth and have identified pp60$^{c\text{-}src}$ as a target for PTPα action.

The in vivo dephosphorylation of Tyr 527 of $pp60^{c\text{-}src}$ in PTPα-overexpressing cells and the cellular association of both proteins with membranes[33], when coupled with the ability of purified PTPα to dephosphorylate the same site of $pp60^{c\text{-}src}$ in vitro, suggests that $pp60^{c\text{-}src}$ may be a direct substrate of PTPα. Since PTPα is widely expressed and is particularly abundant in brain[21, 34] while $pp60^{c\text{-}src}$ is expressed in most cells and at high levels in neural tissue[35], PTPα may function as an in vivo regulator of src kinase activity in normal cells.

TABLE 1. Growth characteristics of parental, control and PTPα-overexpressing cells.

cells	Saturation density (cells/cm^2)	cell proliferation at 0.1% FCS	colonies in soft agar[c] 10% FCS	colonies in soft agar[c] 0% FCS	tumours in nude mice (latency)[d]
REF	ND[a]	No	0	0	0/5
REF-neo	1.2×10^5	No	0	0	0/4
REFα-7	$>1.5 \times 10^6$[b]	Yes	217 ± 20	416 ± 11	4/4 (5-6d)
REFα-21	$>1.5 \times 10^6$	Yes	420 ± 18	789 ± 42	4/4 (5-6d)
REFα-29	$>1.5 \times 10^4$	Yes	781 ± 1	456 ± 28	4/4 (5-6d)

a ND, not determined

b No apparent saturation density was observed with α-7, α-21 or α-29 cells. Proliferation continued to the point where fresh medium was required every 3-5 hours and eventually these cells lifted off the dishes in sheets.

c 400 cells were added to each 6 cm petri dish and colonies were counted after three weeks. Counts are the average of triplicate experiments ± standard error.

d Each mouse was injected subcutaneously at one site on the back with 2×10^5 cells.

EXAMPLE 2

Effect of sodium orthovanadate on proliferation of PTPα-overexpressing and control (REF-neo) cells

1. Cells were grown on 5 cm petri dishes in DME medium with 10% FCS for 48 h. After 48 h of growth, each dish of α-7, α-21, and α-29 cells contained about 0.4×10^5 to 0.6×10^5 cells, and each dish of REF-neo cells contained 1.8×10^5 cells. The medium was discarded, and fresh medium containing 10% FCS and the indicated concentration (below) of sodium orthovanadate was added to the cells. After 48 h of culture in this medium the cells from two replicate dishes were harvested and counted. The results are shown in Table 2 below. They demonstrate that vanadate inhibits the proliferation of the PTPα-overexpressing cells (α-7, α-21, α-29) in a dose-dependent manner while having relatively little effect on that of the control cells (REF-neo).

TABLE 2

Vanadate conc (μM)	Number of cells/5 cm dish			
	REF-neo	α-7	α-21	α-29
0	1.20×10^6	1.80×10^6	1.60×10^6	1.72×10^6
5	1.10×10^6	1.72×10^6	1.42×10^6	1.62×10^6
30	1.04×10^6	1.41×10^6	7.00×10^5	7.20×10^5
60	9.90×10^5	1.18×10^6	3.70×10^5	4.70×10^5
120	7.60×10^5	5.80×10^5	1.60×10^5	2.10×10^5

2. Cells (control cells = REF-neo, PTPα-overexpressing cells = α-21) were grown on 5 cm petri dishes in DME medium with 10% FCS for 48 h. The medium was discarded, and fresh medium containing 10% FCS and the indicated concentration (below) of sodium orthovanadate was added to the cells. After 72 h of culture in this medium the cells from two replicate dishes were harvested and counted. The results are shown in Table 3 below and are essentially the same as in 1 above.

TABLE 3

Vanadate conc (μM)	Number of cells/5cm dish REF-neo	α-21
0	1.84×10^6	1.38×10^6
5	1.90×10^6	1.20×10^6
30	1.80×10^6	4.80×10^5
60	1.60×10^6	2.60×10^5
120	6.70×10^5	1.76×10^5

3. PTPα-overexpressing cells (α-21) were grown for 48 h (to about 60% confluency) in DME medium containing 10% FCS. The medium was removed and replaced with medium containing the indicated concentration of sodium orthovanadate (below) and either 10% (sets #1 and #3) or 0.1% FCS (set #2). The cells were cultured for 48 h and counted (sets #1 and 2), or the medium was removed from set #3 and replaced with medium containing 10% FCS without sodium orthovanadate and cultured for another 48 h before counting. The results are shown in Table 4 below and demonstrate that the proliferation of α-21 cells grown in either 10% or 0.1% FCS is similarly affected by

vanadate in a dose-dependent manner and that vanadate is not irreversibly toxic to these cells since they will resume proliferation once vandate is removed from the medium as may be appreciated by comparison of the cell numbers in sets #1 and 3.

TABLE 4

Vanadate conc (μM)	set #1 (+10% FCS)	set #2 (+0.1% FCS)	set #3
0	1.62×10^6	1.52×10^6	5.12×10^6
5	1.30×10^6	1.18×10^6	2.90×10^6
30	5.00×10^5	5.40×10^5	1.46×10^6
60	3.20×10^5	3.80×10^5	9.60×10^5
120	1.80×10^5	2.00×10^5	5.20×10^5

Example 3: Effect of sodium orthovanadate on the colony forming ability of PTPα-overexpressing α-21 cells.

The α-21 cells were grown in soft agar in the presence of the indicated concentration of sodium orthovanadate (below) on duplicate dishes. After 9 days the colonies were counted on each dish and the approximate size of the colonies noted. The results are shown in Table 5 below and demonstrate the reduced ability of the PTPα-overexpressing cells to form colonies in soft agar in the presence of vanadate.

TABLE 5	Number of colonies			
Vanadate conc (μM)	Exp 1	Exp 2	Average	Colony size
0	1650	1461	1556	+++
5	1127	1392	1260	+++
10	1458	934	1196	+++
20	692	641	667	++
40	312	271	292	+
80	90	73	82	+/-

Example 4: Determination whether vanadate can block or inhibit tumourigenesis caused by REF α-21 PTPα-overexpressing cells.

The α-21 cells were grown in DME medium containing 10% FCS and then in DME medium with 0.1% FCS in the presence of 50 μM sodium orthovanadate for 48 h. The cells were then harvested by trypsinisation, centrifuged to pellet the cells, and the cell pellet resuspended in DME containing 50 μM sodium orthovanadate. Other α-21 cells were grown and prepared in a similar manner, except in the absence of sodium orthovanadate throughout. The resuspended cells (plus or minus vanadate) were subcutaneously injected into nude mice (2×10^6 cells/mouse, 4 mice for each treatment), and nine days later the tumour size was measured. The results below show that tumours arising from cells grown and injected in the presence of vanadate are on average 2.6 times smaller than those arising from cells grown and injected in the absence of vanadate.

cells + vanadate	tumour size	average tumour size
mouse 1:	0.30 x 0.8 cm = 0.24 cm^2	0.618 cm^2
mouse 2:	0.70 x 1.5 cm = 1.05 cm^2	
mouse 3:	0.40 x 0.7 cm = 0.28 cm^2	
mouse 4	0.75 x 1.2 cm = 0.90 cm^2	

cells - vanadate	tumour size	average tumour size
mouse 5:	0.80 x 1.4 cm = 1.12 cm^2	1.603 cm^2
mouse 6:	0.75 x 2.2 cm = 1.65 cm^2	
mouse 7:	0.90 x 1.8 cm = 1.62 cm^2	
mouse 8:	0.88 x 2.3 cm = 2.02 cm^2	

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CLAIMS

1. An inhibitor of protein tyrosine phosphatase α for use in the tr atment of a tumour exhibiting an elevated level of $pp60^{c\text{-}src}$ kinase activity.

2. An inhibitor according to claim 1, in which the tumour is human colon carcinoma.

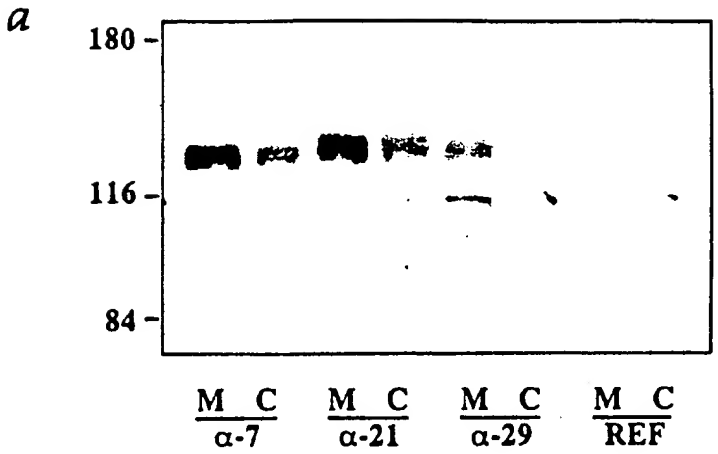
3. An inhibitor according to claim 1 or 2, which is a chemical compound.

4. An inhibitor according to claim 1 or 2, which is an antibody.

5. An inhibitor according to claim 1 to 2, which is a cDNA encoding a mutant protein tyrosine phosphatase α.

6. Use of an inhibitor of protein tyrosine phosphatase α in the manufacture of a medicament for use in the treatment of a tumour exhibiting an elevated level of $pp60^{c\text{-}src}$ kinase activity.

Fig.1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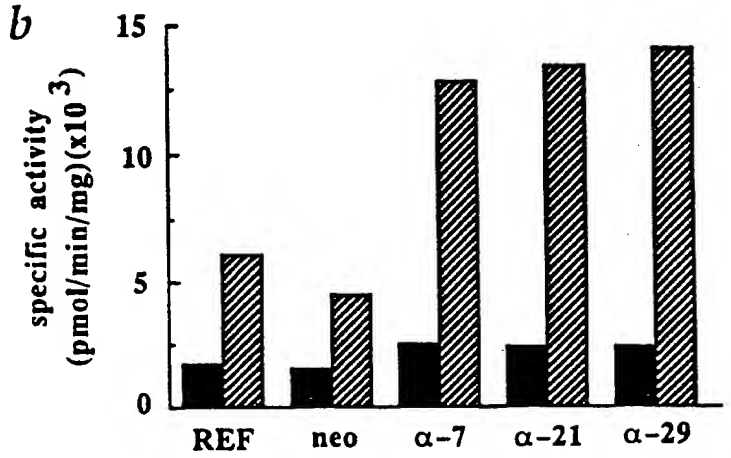
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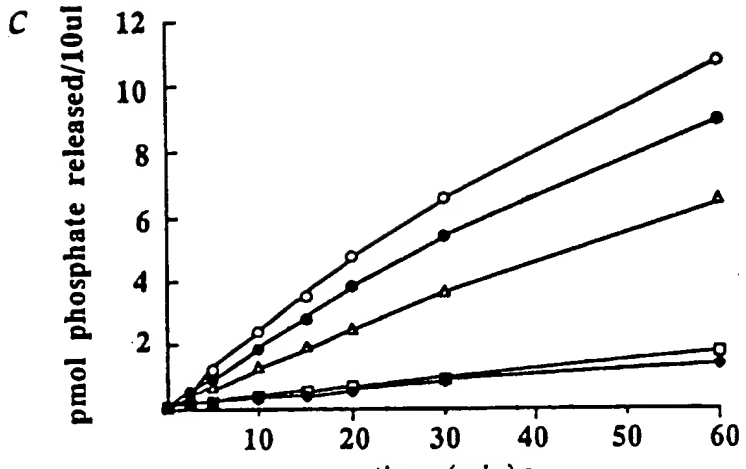
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60
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Fig. 2

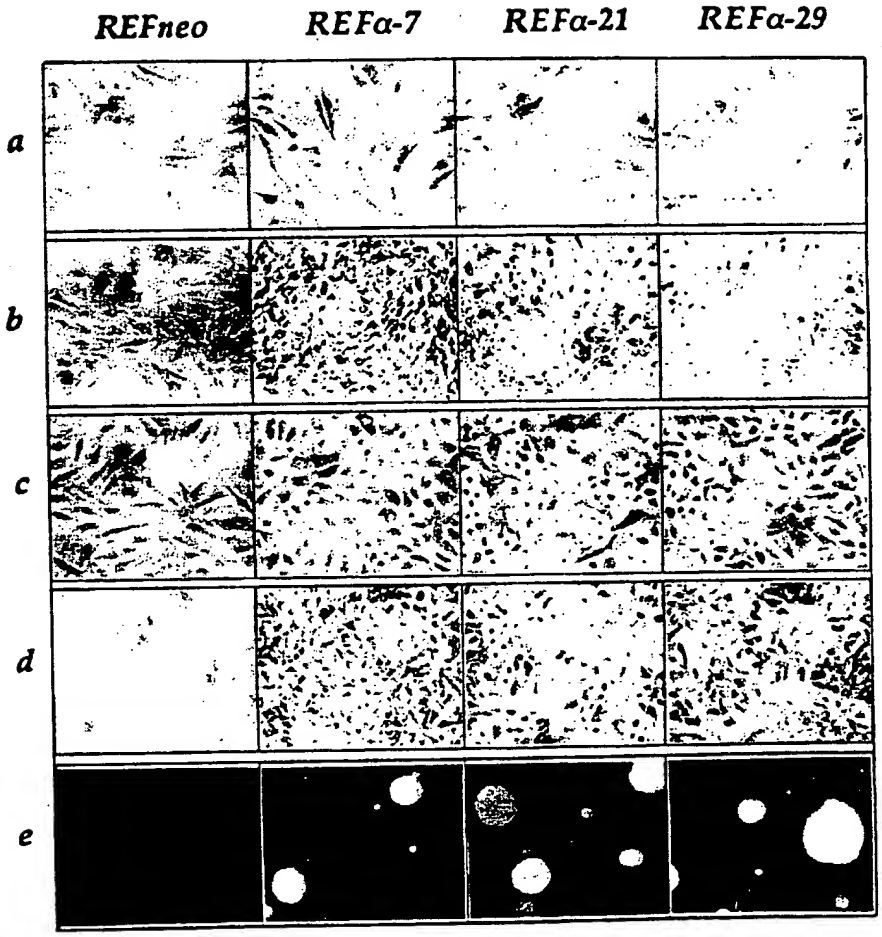
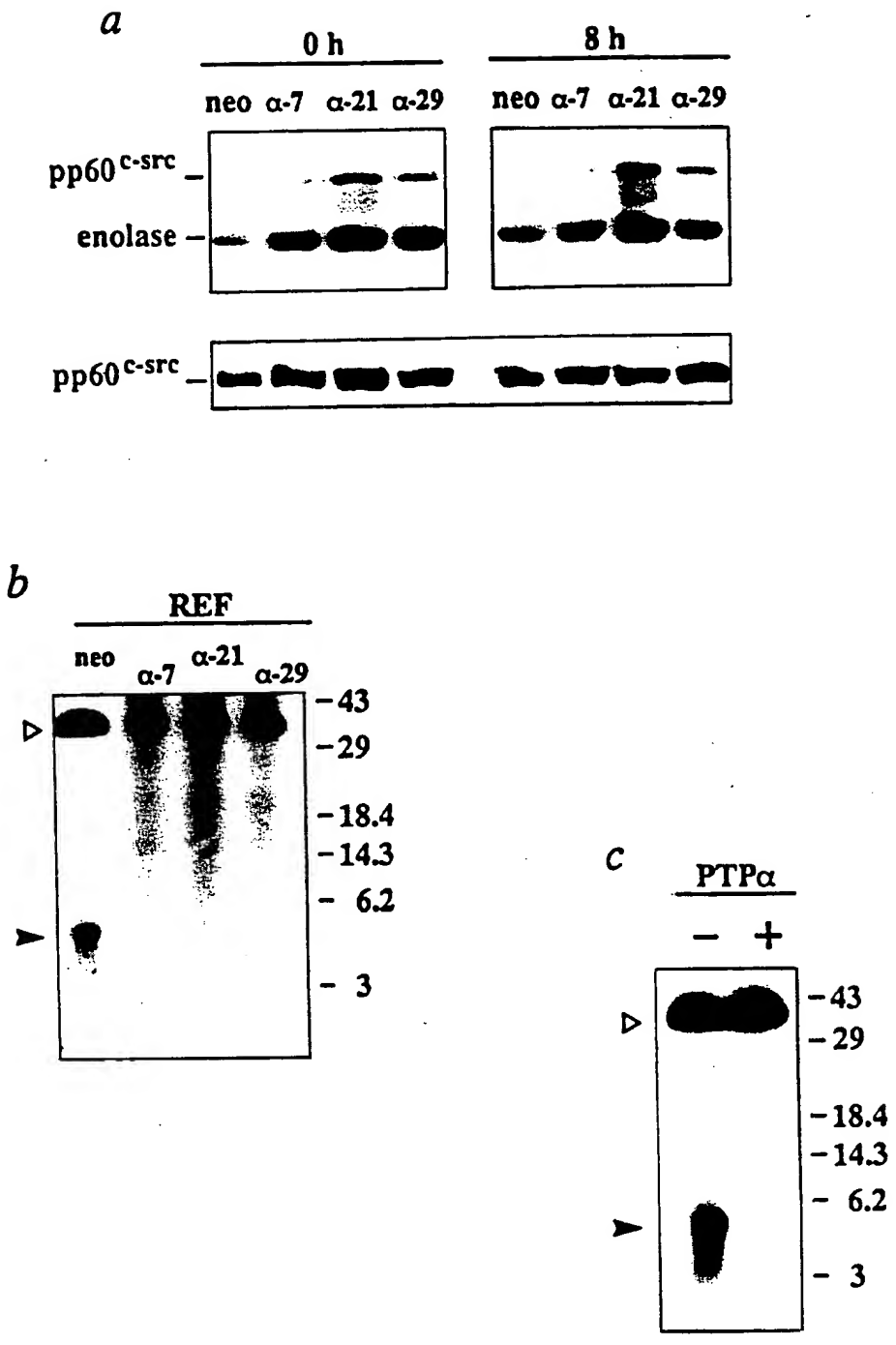


Fig. 3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INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 93/00285

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K33/24; A61K39/395; A61K31/70; //A61K48/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	WO,A,9 113 989 (WASHINGTON RESEARCH FOUNDATION) 19 September 1991 see page 2, line 8 - page 3, line 16; claims ---	1-6
Y	WO,A,9 201 050 (NEW YORK UNIVERSITY) 23 January 1992 see page 4, line 32 - page 5, line 15 see page 24, line 28 - page 25, line 2 see page 33, line 30 - page 34, line 12; claims ---	1-6
A	EP,A,0 245 979 (MERCK & CO., INC.) 19 November 1987 see the whole document --- -/--	1-6

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 OCTOBER 1993	15. 10. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NOOIJ F.J.M.

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EMBO JOURNAL vol. 10, no. 11, November 1991, OXFORD, GB pages 3231 - 3237 Y. WANG ET AL. 'The receptor-like protein tyrosine phosphatase HPTPalpha has two active catalytic domains with distinct substrate specificities.' see the whole document	1-6
P,A	NATURE vol. 359, no. 6393, 24 September 1992, LONDON, GB pages 336 - 339 X. ZHENG ET AL. 'Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase.' see the whole document	1-6

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CA 9300285
SA 76595

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO-A-9201050	23-01-92	AU-A- 8412891	04-02-92
		EP-A- 0538401	28-04-93
EP-A-0245979	19-11-87	US-A- 4710469	01-12-87
		JP-A- 63022186	29-01-88

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